

SYMPOSIUM ON VIRUS DIAGNOSIS IN THE PUBLIC HEALTH LABORATORY¹

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Recent advances in the techniques of growing viruses have greatly simplified the procedures of isolation and identification of the causative agents of virus diseases as well as the performance of various serological tests for antibodies in paired sera from cases. Two of the most important developments in this field have been the incorporation of suitable antibiotics into laboratory media for the control of bacterial contaminants and the widespread use of tissue culture techniques made possible by recent demonstrations of the adaptive tropism of many viruses which previously could only be grown in expensive laboratory animals. The resultant simplification of technical procedures makes it possible for the smaller, less specialized laboratories such as those of most public health departments to perform tests never before attempted in such laboratories and provides the necessity and impetus for the present symposium.

Morris Schaeffer opened the symposium discussing general considerations in the virus diagnosis. He brought out the fact that dramatic advances in virologic techniques have made the rare and highly specialized virus laboratory more common and readily applicable to routine diagnostic procedures. Local requirements should determine the types of services that a state, municipal or hospital laboratory could perform proficiently and economically. Infrequent tests may be provided by a regional or central laboratory which could also serve as a consultant and reference laboratory.

¹ This symposium was held at the Fifty-seventh General Meeting of the Society of American Bacteriologists in Detroit, Michigan, on April 30, 1957. Participants were: Morris Schaeffer, *Communicable Disease Center, Montgomery, Alabama*; Maurice R. Hilleman, *Department of Respiratory Diseases, Walter Reed Army Institute of Research, Washington, D. C.*; Gilbert Dalldorf, *Department of Health, State of New York, Albany*; N. R. Grist, *Virus Laboratory, Ruchill Hospital, Glasgow, Scotland*.

Improvements in antigen production now permit successful utilization of the complement fixation test as a diagnostic tool for the majority of viral and rickettsial diseases. With broader application of the hemagglutination-inhibition test and adaptation of tissue culture methods to serum neutralization tests, routine serologic services, previously prohibitive, are now practicable in most laboratories. Although embryonate eggs, suckling mice, adult mice, and other animals are occasionally mandatory for virus isolation, tissue culture methods are rapidly supplanting these and placing virus isolation and identification procedures within the scope of the average laboratory.

Problems still confronting and deterring the virus diagnostic laboratory are: (a) availability of personnel capable of obtaining reproducible results and interpreting them; (b) availability of standardized diagnostic antigens and antisera; (c) complicated requirements for the collection, preservation and transportation of certain specimens; and (d) the relatively long interval frequently elapsing between submission of specimens and receipt of a final report, which is oftentimes discouraging to the physician.

Progressive advances in electron microscopy, fluorescent antibody techniques, and immunochemistry, as well as in other fields, optimistically promise further simplification and more rapid diagnostic procedures in the near future.

Laboratory diagnosis of viral infections of the respiratory tract was discussed by Maurice R. Hilleman. The respiratory tract, by virtue of its being a hollow organ which is in continuous contact with the atmosphere, is subject to repeated exposure to and attack by a wide variety of microorganisms. Important among these are the viruses which comprise a vast group of known or presently unknown entities which infect the respiratory system. Even though attacked by a diversity of microorganisms, the response of the host to such invasion is singularly limited in variety. Basically, the host response is confined

to fever and constitutional symptoms together with localizing signs and symptoms referable to tissue damage and to inflammation of the respiratory tract. The signs and symptoms in the individual patient vary with the severity of the infectious process and the particular anatomical sites involved. Unfortunately, from the clinical diagnostic viewpoint, many different agents causing respiratory disease may induce essentially the same change in the host resulting in similar clinical findings. As a result of the paucity in differential signs and symptoms in the patient, it is essentially impossible to determine the specific causative agent on clinical grounds alone and the clinician who seeks an etiological diagnosis must rely upon the diagnostic laboratory.

The specific laboratory diagnosis of viral respiratory disease, as in most other viral and rickettsial illnesses, rests on two basic principles. The first is the recovery and identification of the responsible agent. The second is the demonstration of a significant increase in amount of specific antibody against the agent during convalescence from the illness. It is a corollary, therefore, that specific etiological diagnosis must await the discovery of the agent itself and the development of *in vivo* and *in vitro* methods whereby the agent and its antibodies may be detected.

For certain of the respiratory illnesses, laboratory diagnostic methods have been long established and may be considered textbook procedures. This includes tests for influenza A, B, and C, psittacosis-lymphogranuloma venereum group virus infections, Q fever, and the Coxsackie-caused respiratory diseases, *i.e.*, epidemic pleurodynia and herpangina. More recently, notable advances have been made in etiologic discovery in other areas of the respiratory disease picture, largely as a result of application of human cell tissue culture to the respiratory disease problem. The discovery by Hilleman and his associates and by Rowe and others of the new family of adenoviruses has permitted elucidation of a group of syndromes occurring primarily in newly recruited soldiers and including undifferentiated acute respiratory disease (ARD), nonstreptococcal exudative pharyngitis, atypical pneumonia (cold-agglutinin negative) and pharyngoconjunctival fever.

The Sendai agent, a virus of the myxovirus group which was first recovered by Kuroya and his co-workers from a fatal case of pneumonitis in a newborn, has been shown by Jensen *et al.*

and by White *et al.* to infect commonly the human population but to be, apparently, a relatively infrequent cause of respiratory illness. Another new myxovirus, the CA (croup-associated) virus of Channock or laryngotracheobronchitis of Morgan *et al.* has been shown to be a frequent cause of croup with occasional pneumonitis in infants.

Advance in the area of the atypical pneumonias has also been made. Morris *et al.* recently reported the recovery of a new virus, the CCA or chimp coryza agent, from chimpanzees in an epizootic of coryzal illness. Early findings by Channock suggest that this new agent may be associated frequently with pneumonitis in children. The recent work by Liu and others, employing the fluorescent staining procedure, have presented serological evidence of an etiologic relationship of Eaton's egg-propagated Mac virus to another kind of atypical pneumonia, specifically, that associated with the development of cold agglutinins. It is hoped that irrefutable evidence will be forthcoming so that the etiology of this elusive disease entity can be definitively established.

Progress in the elucidation of etiology of mild upper respiratory illness was made by Pelon and associates who recovered a new agent, designated 2060, in monkey kidney tissue cultures. Price has described recovery of what appears to be this same agent from cases of mild coryzal disease in Baltimore.

The recent discoveries of new viral respiratory disease agents has added an exciting chapter to our knowledge of the respiratory illnesses and has expanded the diagnostic spectrum whereby the etiology in individual cases may be determined. It seems safe to conclude, however, that only a small portion of the total aggregate of respiratory disease agents has been uncovered. It is also reasonable to expect that years will pass before the individual viruses have been defined and assigned their proper ranks of importance as disease-producing entities.

Neurotropic virus diagnosis was described by Gilbert Dalldorf. Some years ago public health laboratories worried about the shrinking importance of their work. Now it is found that there are many opportunities for them in their traditional field of infectious diseases. This is very true of the neurotropic viruses, of which only one, rabies, has characteristically been a responsibility of public health laboratories.

Rabies remains a serious problem. Diagnosis

is best done in central laboratories since experience is essential for reliable histologic diagnosis. Since direct examination should be fortified by animal tests, a proper organization would provide a proper supply of mice and a mouse colony is desirable. This is true not only to provide stock mice, the latent virus infections of which are known, but also animals of various ages. In rabies diagnosis immature mice are especially valuable in searching for attenuated strains of rabies virus.

With a suitable mouse colony provided, tests for lymphocytic choriomeningitis may easily be added and, since the small outbreaks of choriomeningitis that occur from time to time are commonly associated with latent infection in house mice, there are typical public health opportunities for the control of infectious diseases in the case of choriomeningitis.

Immature mice provide the simplest and a very satisfactory test method for herpes virus. In herpes, complement fixation tests, using egg membrane antigen, are also very useful.

The arthropod-borne encephalitides are a proper interest of public health laboratories and, while these diseases have not occurred in New York, tests for them by means of complement fixation tests are regularly performed. It has been found worthwhile to prepare antigens in the laboratory. The investment made in the antigen preparation has been worthwhile and it is also recommended as a proper function of public health laboratories.

The bulk of neurotropic virus diseases including poliomyelitis, Coxsackie and ECHO infections are enteric infections and here the public health laboratory has a large and growing responsibility since these infections are not only common but are apparently increasing in importance. In this field, tissue culture provides the most useful technique although immature mice have an essential role. Tissue culture is an effective way of identifying the viruses and may become important in recognizing carriers and in sanitation. It also provides the simplest means of testing sera for antibodies. The present limitation to laboratory procedures in the enteric virus field is due to the variety and complexities of the enteric virus population and much study will be required before the agents that are already known are properly evaluated. A great deal needs to be done. Reference laboratories will be needed, standard reagents and typing sera. These are all proper functions of public health laboratories that might

be added to the preparation of antigens for serologic tests for those viruses previously mentioned and all of which should be made available to hospital laboratories in the areas served by the public health laboratory.

Other enteric viruses remain that are still beyond our skills. The nonbacterial forms of gastroenteritis and hepatitis are outstanding examples.

The laboratory diagnosis of viscerotropic and dermatropic viruses was presented by N. R. Grist. He pointed out that well-established tests are available for the diagnosis of yellow fever by virus isolation or by serological tests for antibody. Similar tests are possible for Rift Valley fever, Colorado tick fever, and dengue. Recent observations in tissue culture give hope that specific diagnostic tests may become available for infectious hepatitis.

Although not generally available as a routine test, tissue culture now provides a basis for the specific diagnosis of measles, rubella, varicella, zoster, and foot-and-mouth disease. Inoculation of animals may establish the diagnosis of contagious echthyma ("orf") and foot-and-mouth disease. Complement fixation reactions have been obtained with material from the lesions of molluscum contagiosum and contagious echthyma.

Valuable routine tests are available for smallpox, vaccinia, cowpox, and herpes-simplex infections, of which atypical manifestations may be difficult to recognize by clinical examination alone. Microscopic examinations of stained smears from the bases of early lesions may show the presence of elementary bodies of smallpox, vaccinia, or cowpox; characteristic multinucleated giant cells are found in lesions of herpes, varicella and zoster. The complement fixation test is a sensitive indicator of pox-group antigen in material from the skin lesions of smallpox, vaccinia, and cowpox; a positive reaction with serum from the pre-eruptive stage of smallpox used as antigen suggests a fatal prognosis. By complement fixation, antigens of herpes simplex virus may also be detected in vesicle fluid. Chorioallantoic egg inoculation is a highly sensitive method of isolation of the viruses of herpes and the pox group. Specimens for this purpose may be taken from the skin or other lesions; smallpox virus is sometimes demonstrable in blood during the early stages of infection. The characteristic appearances of the focal lesions produced in the egg provide a convenient method

of provisional identification and differentiation of the viruses of variola, vaccinia, cowpox, and herpes simplex. For diagnostic isolation of these viruses, the chick embryo has supplanted the rabbit and gives practical advantages over tissue culture. Intraperitoneal inoculation of suckling mice is a more sensitive method of isolation of a few strains of herpes which are undetected by egg inoculation.

Serological diagnosis of smallpox may be attempted by complement fixation, hemagglutination-inhibition or neutralization tests. The antigens involved are common to the viruses of variola, vaccinia, and cowpox, and previous vaccination renders interpretation of the tests difficult. In unvaccinated persons antibodies generally become demonstrable in the second week of illness, but an earlier diagnosis can usually be made by the other types of tests.

Complement fixation and neutralization tests are useful in cases of primary infection with herpes virus, but are unhelpful in secondary and recurrent infections of the majority of persons who already possess antibody.

Virus isolation is the most sensitive laboratory test for smallpox and may be attempted at any stage of the disease, but requires from two to three days for performance. The microscopic test takes less than an hour but gives only a provisional diagnosis and is useful only in the pre-pustular stages of illness. Complement fixation tests for virus antigen or antibody give their results in 24 hr, but the tests for antibody may be difficult to interpret and may not become positive until the late stages of illness. For emergency diagnosis of smallpox, it is helpful to perform several of the available kinds of tests.